Field Captures of Wild Melon Fly (Diptera: Tephritidae) with an Improved Male Attractant, Raspberry Ketone Formate

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ABSTRACT Field-trapping evaluations of the new male attractant, formic acid 4-(3-oxobutyl) phenyl ester (raspberry ketone formate [RKF]) were conducted in Hawaii with wild populations of melon flies, *Bactrocera cucurbitae* Coquillett (Diptera: Tephritidae), to determine its activity in the field and to evaluate new plastic matrix formulations. All tests were compared with the standard melon fly attractant 4-(4-acetoxyphenyl)-2-butanone (cuelure [CL]), which is the attractant of choice for detection programs aimed at melon fly and other cuelure-responding *Bactrocera* fruit flies. Results of these tests over a range of doses on cotton wicks showed that at a 1-g dose raspberry ketone formate was 1.5–2 times more attractive compared with cuelure for up to 11 wk in the field. Lower doses applied on cotton wicks were less active, presumably due to hydrolysis of RKF to raspberry ketone. Raspberry ketone formate embedded in a plastic plug formulation also was field tested, and it was shown to be more attractive to male melon fly compared with cuelure. The use of this new attractant in control and detection programs is discussed.

KEY WORDS attractants, melon fly, raspberry ketone formate, semiochemicals, *Bactrocera*

The melon fly, *Bactrocera cucurbitae* Coquillett (Diptera: Tephritidae), is a serious economic pest on cucurbit crops, such as pumpkin, *Cucurbita maxima* Duch; zucchini squash, *Cucurbita pepo* L.; cucumber, *Cucumis sativas* L.; and melons (*Cucumis* spp.). The melon fly was first introduced into Hawaii in the late 1800s (Back and Pemberton 1917), and it rapidly spread throughout the Hawaiian Islands. Globally, its distribution includes Africa, India, Southeast Asia, and other Pacific islands (White and Elson-Harris, 1992, Kuba et al. 1996). *B. cucurbitae* can cause extensive damage due to larval feeding and tunneling in the fruit, stems, and blooms of the host.

Melon fly is considered a federal quarantine pest due to its highly invasive nature and the threat of establishment into areas of the continental United States and overseas (e.g., New Zealand, Japan, and Australia), where it currently is not established. Detection trapping programs have been established for melon fly by using 4-(4-acetoxyphenyl)-2-butanone (cuelure [CL]), which is the currently accepted international standard for this species and other related cuelure-attracted *Bactrocera* species (USDA–APHIS–PPQ 1991, IAEA 2003). Each year, thousands of traps are deployed to detect introductions of this pest and other tephritid fruit fly species.

Some of the first attractants used for melon fly were anisylacetone and raspberry ketone (RK) (Metcalf and Metcalf 1992). In an effort to find a more effective lure, several derivatives of raspberry ketone were tested. The acetate derivative of raspberry ketone was discovered to be more attractive than RK to melon flies in the field, and it was named cuelure (Beroza et al. 1960). For the past 40 yr, this molecule has been used worldwide in detection trapping programs for melon fly and other CL-responding *Bactrocera.*

In the 1990s, laboratory and preliminary field studies showed that the formate ester of raspberry ketone, formic acid 4-(3-oxobutyl) phenyl ester (RKF), was more attractive to melon flies than raspberry ketone or cuelure (Metcalf and Mitchell 1990, Metcalf and Metcalf 1992). Surprisingly, further research on RKF or more effective replacements for CL then nearly disappeared for ≈10 yr. Thus, although the superiority of RKF over CL in attracting melon fly had been reported, no further attempt was made to develop the attractant as a replacement for CL. Most recently, Casana-Giner et al. (2003b) reported the synthesis of raspberry ketone formate and preliminary field responses to RKF compared with CL. These studies again showed that RKF was more attractive than CL, but they identified the hydrolysis of RKF to RK as a possible problem with the molecules's longevity. Hydrolysis experiments of RKF in the laboratory showed that hydrolysis decreased with increased doses of RKF

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(Casana-Giner et al. 2003b). In a separate study, several fluorinated and silylated analogs of RK also were synthesized and tested (Casana-Giner et al. 2003a), but they were not any more attractive than CL in field bioassays.

This article reports the results of experiments to evaluate the responses of wild melon flies to RKF and CL presented on a cotton wick and in a proprietary plastic matrix. In addition we wanted to determine whether incorporating RKF and CL into the plastic matrix would affect longevity of RKF in the field and possibly reduce the reported hydrolysis of RKF to RK. However, we did not attempt to directly measure hydrolysis of RKF to RK in this study, but we used field attractancy and efficacy compared with CL to determine effectiveness of the lure. Several doses were tested on cotton wicks to try to minimize the effects of hydrolytic instability on the longevity of RKF, which had been reported previously (Metcalf and Metcalf 1992, Casana-Giner et al. 2003b). Finally we evaluated RKF and CL in the plastic matrix at doses used in detection programs on wild fly populations.

Materials and Methods

Insects. Tests were conducted with wild populations of melon flies in the borders of papaya, *Carica papaya* L., fields in the Kapoho area on the island of Hawaii. Established populations in this area are due to high infestation rates of melon flies found in fallen papaya fruit on the ground (Liquido 1991).

Treatments. RKF (97%) was synthesized at the USDA–ARS–CAIB Laboratory in Beltsville, MD (Casana-Giner et al. 2003b). Cuelure (95%) was purchased from Orsynex, Inc. (Columbus, OH). Scentry Biologicals (Billings, MT) provided RKF and CL formulated in proprietary plastic matrices that held the liquid in place and allowed for evaporation of the liquid at controlled release rates.

Twenty milligrams, 160 mg, and 1 g of RKF, CL, and a solvent only blank control were applied on 1.2–2.5-cm cotton dental wicks secured inside a plastic basket (Agrisense, Palo Alto, CA). Raspberry ketone formate (2 g of active ingredient [AI]) and cuelure (2 g of [AI]) plastic plugs (total weight 3 g) also were tested against 2 g of CL and 2 g of RKF on two 3.5-cm cotton wicks placed in plastic mesh baskets. A toxicant (Hercon Vaportape II pest strip, Hercon Environmental, Emigsville, PA) also was placed in each trap to kill the flies upon entry into the trap. Treatments were placed in large plastic bucket type traps (20 cm in height by 20 cm in diameter; KYD Distributors, Honolulu, HI). Four 2.5-cm-diameter holes were drilled around the bucket 3 cm from the top of the bucket. Several 2-mm holes were drilled in the bottom of the bucket for drainage of any accumulated rainwater. The wick, enclosed in a plastic mesh basket, and the Vaportape were hung from a wire hanger on the underside of the bucket cover.

Experiments. Tests were conducted at multiple sites located on the borders of papaya fields in Kapoho on the island of Hawaii. Maximum field temperatures ranged from 22 to 26°C. Traps were hung in a randomized block design on the borders of papaya fields ≈20–30m apart. Traps were serviced once a week. In each case the lures were allowed to weather over the indicated test period. At each service, flies were removed from traps, counted and recorded. In the first series of experiments, test periods ranged from 4 to 12 wk. If required, the Vaportape was replaced every 6 wk. In the first series of studies, tests were conducted in fields with wild melon fly populations where bucket traps containing cotton wicks loaded with either 20 mg, 160 mg, or 1 g of RKF were compared with the corresponding dose of CL and a solvent control placed in 10 blocks. In a second study, baited traps containing 2 g (AI) of RKF and CL plugs and wicks were hung 20–30 m apart in four blocks. The test period for this study was 32 wk. Only male melon flies captured were counted and recorded due to the absence or low numbers of female flies captured.

Data Analysis. Mean male trap captures were analyzed using PROC GLM followed by a Tukey's honestly significant difference (HSD) test for mean separation. The data were transformed by square root (capture + 0.5) before the analysis. Significant differences were determined at the $P < 0.05$ level. Analysis was run on SAS version 8.2 (SAS Institute 1990). Mean flies ± SE per trap per day are presented.

Results

In the field tests, both RKF and CL readily captured flies, whereas few flies were captured in the control traps. Captures at the 20-mg dose were significantly greater in RKF-baited traps than CL-baited traps for the first 2 wk ($F = 79.95$, $F = 45.52$, $P < 0.0001$). In weeks 3 and 4, there were no significant differences in trap captures (Fig. 1).

At 160-mg dose, traps baited with RKF captured significantly more male melon flies than CL for the first 3 wk of testing ($F = 31.25$, $F = 51.97$, $P < 0.0001$). At 4–6 wk, there were no significant differences in male fly capture between RKF and CL (Fig. 2).

Traps baited with 1-g dose of RKF captured significantly more male melon flies compared with CL for up to 11 wk in the field ($F = 292.94$, $P < 0.0001$) (Fig. 3).

Field tests conducted with RKF and CL formulated in a plastic matrix showed that the traps baited with 2-g (AI) RKF plug captured significantly more male melon flies compared with all other treatments. RKF (2 g) on a wick and the cuelure plug (2 g) were not significantly different from each other, but both captured significantly more flies than 2 g of CL on a wick, which was significantly more attractive than the control (Table 1).

Discussion

The results of the dose–response field tests with RKF on a wick showed that at the lower doses, RKF captured significantly more melon flies compared with

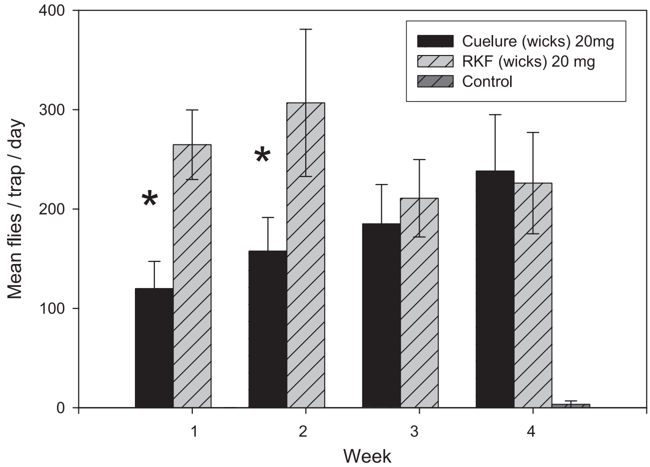

Fig. 1. Trap captures of wild melon flies to male attractants in open field tests in Kapoho. Captures expressed as mean flies per trap per day. Symbols indicate significant differences at $P < 0.05$. *, $P < 0.05$.

the same dose of CL for 2 wk in the field, after which it is not significantly different from CL. These results with wild fly populations support our earlier work on the attractiveness and efficacy of RKF in the field by using released laboratory-reared flies (Casana-Giner et al. 2003b). At an increased 160-mg dose, RKF was significantly more attractive than CL for the 3 wk reported. The eightfold increase (20–160 mg) in dose did not increase attractiveness or longevity as we had expected. However, at the 1-g doses, RKF baited traps captured significantly more melon flies compared with CL for a longer period of up to 11 wk. These data indirectly support our earlier finding that increased doses of RKF reduce the effects of hydrolysis and extend attractiveness compared with CL at higher doses. That after 2, 3, or 11 wk, RKF still maintains attractiveness equal to CL (on cotton wicks) may indicate a more steady-state condition where the increased inherent activity of RKF over CL is offset by the hydrolytic activity of RKF (or CL) to RK. RK, being less attractive and less volatile than even CL, does not attract significant numbers of flies relative to RKF.

The standard dose of CL used in detection/monitoring traps in fruit fly programs in California, Texas, and Florida is 2–6 g of CL on a cotton wick. When we compared the newer plastic matrix formulations of RKF and CL containing 2 g (AI) with the same amount on the cotton wick, we found that the newer formulations of either attractant on the proprietary matrix was significantly better than on the cotton wick over the 32-wk trapping period (Table 1). More importantly the RKF plug-baited traps caught twice as many flies as the CL plug-baited traps during this period. In a previous study (Casana-Giner et al. 2003b), we showed that hydrolysis of 1 g of RKF from a cotton wick increased up to ≈26% after 50 d of weathering. RKF presented in a plastic matrix improved trap cap-

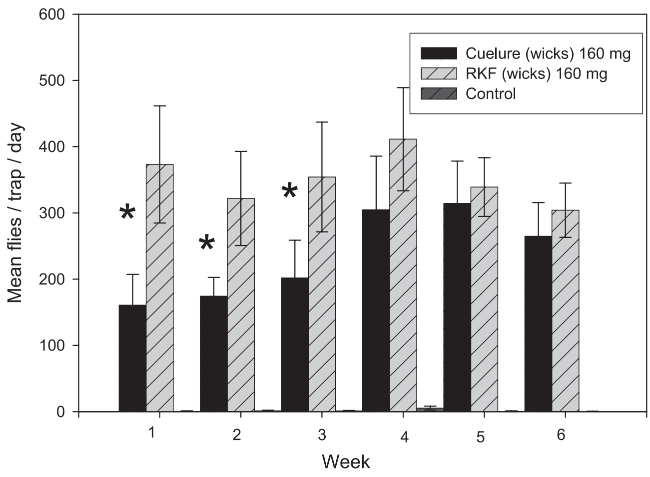

Fig. 2. Trap captures of wild melon flies to male attractants in open field tests in Kapoho. Captures expressed as mean flies per trap per day. Symbols indicate significant differences at $P < 0.05$. *, $P < 0.05$.

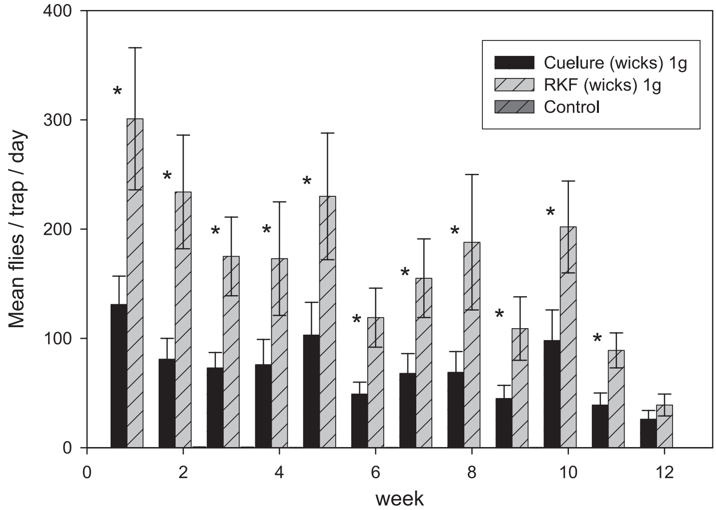

Fig. 3. Trap captures of wild melon flies to male attractants in open field tests in Kapoho. Captures expressed as mean flies per trap per day. Symbols indicate significant differences at $P < 0.05$. *, $P < 0.05$.

ture (and presumably its hydrolytic stability) versus a cotton wick. We hypothesize that this might be due to increased exclusion of water from the matrix. Differences in release rate of the active attractant on the matrix compared with the cotton wick also may help account for the extended longevity of the lure. This active threshold of RKF caused by reducing hydrolytic activity showed increased longevity at the higher doses. Fly captures as measured by flies per trap per day were nearly twice that of CL over the test period (Table 1).

Further tests with varying proportions of RKF, CL, and RK may shed additional insight on how these molecules interact with the environment and the resulting attraction of melon fly to these molecules. Changes in matrix formulation also are being considered as well as specific release rate and hydrolysis studies to determine the precise rate of hydrolysis in a given matrix or formulation. Further field tests will be carried out in other locations with melon fly populations and also with other *Bactrocera* species to determine the optimal formulation of RKF.

The oriental fruit fly, *Bactrocera dorsalis* Hendel was successfully eradicated from Rota (Steiner et al. 1965), Saipan (Steiner et al. 1970), and Okinawa (Koyama et al. 1984) by using the male annihilation technique with the powerful male lure methyl eugenol. Unfortunately, successful male annihilation of the melon fly could not be achieved by trapping only with CL. In 1970, aerial applications of CL were carried out by helicopter over the island of Hawaii. Initial trap captures were reduced by 97%; however, after the spray trials ended, trap captures quickly increased. It was evident that the residues were not lasting as long as desired (Cunningham et al. 1970). Also, male annihilation control of melon fly was demonstrated with CL and a toxicant in semi-isolated populations, but it failed to reduce fruit infestation rates (Cunningham and Steiner 1972). The increased effectiveness of RKF (2× CL) could potentially be used alone or in conjunction with the longer lasting CL to develop effective detection, control, and eradication technologies for CL-responding *Bactrocera* species.

Raspberry ketone formate seems to be a better attractant then the current standard CL at capturing wild melon fly in the field. This increased activity seems to be approximately twice that of CL over 11 wk when formulated into a plastic matrix. Thus, RKF would seem to be the molecule of choice for use in early detection and eradication programs where capturing the first fly or trapping out small or newly established populations is of utmost importance. However, in suppression of established populations the longevity of CL and stability in the field should be considered. The development of plastic matrices for controlled release of RKF may further improve the stability and longevity of this molecule while possibly reducing hydrolytic activity and increasing ease of use.

Table 1. Response of plug and wick treatments in wild populations of melon flies in Kapoho, HI

Treatment	*n*	Mean flies/trap/d ± SE
RKF, plug 2 g	127	210 ± 14a
RKF, wick 2 ml	126	124 ± 16b
Cuelure, plug 2 g	127	97 ± 7b
Cuelure, wick 2 ml	127	59 ± 6c
Control	127	0.5 ± 0.1d

Treatments followed by the same letter are not significantly different at the 0.05 level (PROC GLM, Tukey's HSD; SAS Institute 1990).

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